form an image of the objects onto the corresponding detectors 282. In this configuration, each detector 282 has fewer pixels than other embodiments because each detector covers only one spectral band. In the case where a six-color version of this embodiment is employed, the images on a single detector would appear like the images seen on one zone of the detector illustrated in FIGURE 17. For example, the images seen on the detector configured to receive light in the red part of the spectrum would appear like the right-most zone of FIGURE 17. Since the total number of pixels in each detector is low, these detectors may operate at very high speeds. The embodiment shown in FIGURE 16A has the advantage of being more efficient than other embodiments, because the light from the object only passes through each dichroic filter 277 once. A still further advantage of this embodiment is that each detector 282 can be focused independently for each color thereby simplifying the optical design by removing the constraint of longitudinal color correction. An additional objective lens 48 and slit 52, such as that shown in FIGURE 2C can be incorporated into the embodiment shown in FIGURE 16A to prevent unwanted light from striking one or all of the pixelated detectors.

Fifth Embodiment of Apparatus for Spectral Decomposition and Imaging

FIGURE 16B illustrates another embodiment for spectral decomposition imaging. It is similar to the previous embodiment, however, it has the advantage of reducing the number of imaging lenses required to project an image upon the detectors. In the imaging system of FIGURE 16B, light from beads (or other objects) is focused by an objective lens 281 onto a slit 283. Slit 283 is sufficiently narrow to block light that is not focussed onto the slit by objective lens 281, thereby preventing extraneous light from passing through the slit. Light which has passed through the slit, is collected by a collection lens 284 and then directed to imaging lens 285, both of which are placed before the dichroic filters 286. Each detector 287 is placed at the appropriate position along the optical path to image the object onto the surface of each detector 287. Detectors 287 are placed at the appropriate positions along the optical path to image beads or other objects onto detectors 287. The filters are placed in convergent space with respect to the image of the object and therefore each filter, depending upon its design, may impart astigmatism, coma, spherical and chromatic aberration into the imagery at each downstream detector. Progressively more of each of these aberrations are added by each subsequent filter. In a typical implementation of the present invention, the numerical aperture in the filter space is approximately 0.03. Therefore, if cube substrates are employed for the dichroic filters, coma and astigmatism are negligible while spherical aberration is less than 0.15 waves peak. Longitudinal chromatic aberration is effectively canceled by moving the detectors to the plane of best

focus for their respective color band. Pellicles can also be used in place of the cubes with excellent theoretical optical performance.

If plate substrates are employed for dichroic filters 286, then astigmatism dominates the aberrations. Astigmatism is imparted in the transmitted wavefront through a plate dichroic filter, but can effectively be cancelled by inserting a clear correction plate 360, as shown in FIGURE 16C, of approximately the same thickness, incident angle, and glass type. However, correction plate 360 must be rotated 90 degrees about axis Z with respect to dichroic filter 361. Correction plate 360, and dichroic filter 361 impart an equal but opposite amount of astigmatism in the transmitted wavefront, thereby canceling each other. Therefore, light striking detector 342 is free of astigmatism. This configuration leaves a small amount of residual coma. Yet, the optical performance is very close to the diffraction limit. Note that because light striking a detector 341 is reflected light, as opposed to transmitted light, there is less concern about astigmatism.

Those of ordinary skill in the art will appreciate that the correction plate can be placed in many alternative positions, with adjustments in its thickness, material, and/or angle, relative to the propagation of the light. Note that FIGURE 16C shows only part of an optical system, including only some of the elements employed after a collection lens 340. Of course, the flow cells, optional light sources and additional detectors (see FIGURE 16A) are included in a complete system.

Any of the non-distorting spectral dispersing embodiments can be constructed using an additional objective lens 48 and slit 52, to form a confocal stop arrangement as shown in FIGURE 16B. FIGURE 16B also illustrates that detectors may be placed in either the transmission or reflection paths of dichroic filters. Either of the multiple detector embodiments may constructed such that the detectors receive light transmitted through the dichroic filters, reflected by the dichroic filters or in a combination of transmission and reflection as illustrated in FIGURES 16B.

Note that FIGURE 16B also indicates optional ancillary components that might be beneficially incorporated into imaging systems in accord with the present invention, such as a pumping system, a reservoir for fluid into which encoded beads are entrained, and a reservoir for beads that have already been imaged and read. It should be understood that such components could be similarly incorporated in the other imaging systems of the present invention.

In all spectral decomposition and imaging embodiments, it is anticipated that the magnification is set such that one detector pixel in object space is roughly the size of a single reporter. Given a detector pixel size of 10 microns and a reporter size of one

micron, the magnification would be configured to approximately 10X. Likewise, if the reporter were 0.5 microns, the magnification would be set to approximately 20X.

As disclosed in the above-referenced U.S. Patent No. 6,211,955 and as shown in FIGURES 1 and 4, multiple legs of the spectral decomposition and imaging system may be used to collect images and corresponding signals from multiple perspectives. Two legs are shown in FIGURE 1; however, if epi illumination is applied as shown in FIGURES 3 and 4, four legs may be used to view the beads from each side of the cuvette. Light emitted from the beads may also be collected with objective lenses that can be optically coupled directly to the cuvette to improve the numeric aperture. In addition, each optical leg can image beads at multiple focal planes to improve focus on reporter beads which may be out of focus at a different focal plane.

Pixelated Detection

Pixelated detection employs frame-based CCD image collection, in which a CCD detector views beads in flow, in a freeze frame fashion. This method requires the integration time to be very short to prevent blurring. A short integration time is achieved either with a strobed light source, or a continuous light source combined with a shuttered or gated detector. In either case, the short integration time reduces the signal-to-noise ratio and the ultimate sensitivity of the approach with fluorescence signals. Further, frame-based cameras require time to transfer data out of the pixelated detector, during which no images are acquired, and beads of interest can escape detection. However, these types of detectors are readily available, inexpensive and do not require an accurate knowledge of the velocity of the beads in flow.

Alternative Approach for Pixelated Detection

Another approach useful in bead imaging for pixelated detection also employs frame-based CCD image collection, but does not rely on strobed illumination or shuttered detection to freeze image motion. Instead, a rotating or oscillating mirror (not shown) is used to compensate for bead motion to produce a still image on the detector. This technique may employ continuous illumination, thereby achieving higher levels of sensitivity than strobed systems when analyzing fluorescence. However, this approach requires both an accurate measurement of bead velocity and a very stable fluid pumping system, since the inertia of the mirror prevents compensation for rapid changes in bead velocity.

A Further Technique for Pixelated Detection

Yet another method for pixelated detection uses TDI CCD image collection. In TDI detection, the electronic signal produced within the detector by an incident image is moved down the detector in synchrony with the motion of the image. In this manner,